International Journal of Pharmaceutics xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Spray dried amikacin powder for inhalation in cystic fibrosis patients: A quality by design approach for product construction

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ARTICLE INFO

Article history:
Received 14 May 2014
Accepted 28 May 2014
Available online xxx

Keywords:
Dry powder inhaler (DPI)
Amikacin
Cystic fibrosis
Half-fractional factorial design
Orally inhaled drug product (OIDP)

ABSTRACT

An amikacin product for convenient and compliant inhalation in cystic fibrosis patients was constructed by spray-drying in order to produce powders of pure drug having high respirability and flowability.
An experimental design was applied as a statistical tool for the characterization of amikacin spray drying process, through the establishment of mathematical relationships between six Critical Quality Attributes (CQAs) of the finished product and five Critical Process Parameters (CPPs).
The surface-active excipient, PEG-32 stearate, studied for particle engineering, in general did not benefit the CQAs of the spray dried powders for inhalation. The spray drying feed solution required the inclusion of 10% (v/v) ethanol in order to reach the desired aerodynamic performance of powders. All desirable function solutions indicated that the favourable concentration of amikacin in the feed solution had to be kept at 1% w/v level. It was found that when the feed rate of the sprayed solution was raised, an increase in the drying temperature to the maximum value (160 °C) was required to maintain good powder respirability. Finally, the increase in drying temperature always led to an evident increase in emitted dose (ED) without affecting the desirable fine particle dose (FPD) values.
The application of the experimental design enabled us to obtain amikacin powders with both ED and FPD, well above the regulatory and scientific references. The finished product contained only the active ingredient, which keeps low the mass to inhale for dose requirement.

1. Introduction

Cystic fibrosis (CF) is an autosomal recessive genetic disease in Caucasians. Mutations in the gene cystic fibrosis transmembrane regulator (CFTR) led to a defective chloride ion transport in the airway lumen, causing an abnormal accumulation of viscous mucus. CFTR mutations are grouped into five classes scaled from not synthesized CFTR protein (class I) to partly defective protein production or processing (class V) (Amaral and Kunzelmann, 2007). The most common are the class II mutations (including the prevalent, F508del), which affect about 90% of CF patients. In this class, the misfolded protein is retained at the endoplasmic reticulum, and subsequently degraded in the proteasome, (Ratjen and Döring, 2002; Coffey and Ooi, 2012).

Cystic fibrosis in the lungs often gives rise to chronic pulmonary infections. Bacteria entering the lung and trapped in the mucus layer are difficult to remove through mucociliary clearance. The therapy of CF infections consists of antibiotics administered orally or intravenously (aminoglycosides, macrolides). As the lung is the site of action, inhaled medications are advantageously prescribed (Heijerman et al., 2009; Bruce et al., 2011; Balducci et al., 2014; Colombo et al., 2013). This administration route concentrates the drug at the site of infection, limiting systemic exposure and side effects. However, substantial differences in inhalation administration techniques exist. For instance, nebulizers, mostly used in hospitals and

Abbreviations: AMK, Amikacin; DPI, Dry Powder Inhaler; QbD, Quality by Design; DoE, Design of Experiments; CQAs, Critical Quality Attributes; CPPs, Critical Process Parameters; LOD, Loss on Drying; ED, Emitted Dose; FPD, Fine Particle Dose.

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http://dx.doi.org/10.1016/j.ijpharm.2014.05.055
0378-5173/

outpatient care, are cumbersome for chronic disease management, showing low efficiency and reproducibility. Dry powder inhalers (DPI), activated by patient inspiration effort, are a substantial alternative because of rapid dose administration, high local drug deposition and concentration, and stability (Buttini et al., 2012).

Among the prescribed antibiotics, aminoglycosides bind bacteria ribosomes and inhibit the protein synthesis (Borovinskaya et al., 2007). Gentamicin and amikacin also bind the eukaryote ribosomes (Manuvakhova et al., 2000; Wilschanski et al., 2003). Gentamicin suppressed the G542X premature stop mutation (class II) in a CF transgenic mouse model (Du et al., 2002). This effect was observed *in vivo* at peak serum concentrations well above the level required for antibiotic activity. However, the clinical use of gentamicin for this activity is limited because of its systemic side effects. In alternative, amikacin in mouse model was shown to be safer and more effective than gentamicin in suppressing the CFTR protein premature stop mutation, (Du et al., 2006). A liposomial formulation of amikacin for nebulization has recently been granted orphan drug designation for the treatment of *Pseudomonas Aeruginosa* infections in patients with CF (Li et al., 2008; Clancy et al., 2013). Similarly to other recently marketed pulmonary antibiotics, amikacin dry powder inhaler could represent an advancement of pulmonary therapy in CF patients. Spray dried powders of amikacin and other aminoglycosides with the adjunct sodium stearate in order to increase the respirability and stability of the product have been described (Buttini et al., 2011; Parlati et al., 2009).

The purpose of this work was to construct an amikacin spray dried powder for inhalation that would afford convenience and compliance in usage by cystic fibrosis patients. As spray drying is a multi parametric process, the objective was to systematically gain process understanding of the spray drying method for producing amikacin powders characterized by high drug content, respirability and flowability.

Quality by Design (QbD) is a risk-based, scientific and proactive approach governing the current regulatory framework with regard to pharmaceutical process and product development (www.ich.org). Within this context, an experimental design was applied as a statistical tool for the characterization of the amikacin spray drying process, through the establishment of mathematical relationships between the Critical Quality Attributes (CQAs) of the finished product and the Critical Process Parameters (CPPs) (Korakianiti and Rekkas, 2011; Politis and Rekkas, 2011). In particular, this research was devoted to the understanding of the most influential process parameters affecting the inhalation performance of amikacin spray dried powders. This step opens up the way for optimization exercises capable of revealing the roadmap to further improvement. Finally, in consideration of the typical bulkiness and cohesiveness of the spray dried powders, an agglomeration process for improving the flow characteristics was performed and evaluated.

2. Materials and methods

2.1. Materials

Amikacin sulphate was retrieved by ACS DOBFAR S.p.a., (Milan, Italy) and the surface-active excipient, PEG-32 stearate (Gelucire 48/16), was donated by Gattefossé (Nanterre Cedex, France). All solvents were of analytical grade. Water was purified by reverse osmosis (MilliQ, Millipore, Guyancourt, France).

Hard hydroxy-propyl methylcellulose (HPMC) capsules (size 3) were received from Capsugel (Colmar, France). RS01 Dry Powder Inhaler device (Aerolizer®-like type) was a gift of Plastiape S.p.a. (Osnago, LC, Italy).

2.2. Design of experiments (DoE)

A half-fractional factorial design with 5 factors (n) at two levels with resolution V was employed, allowing for the estimation of the main effects and the two factor interactions. The number of experiments to perform for the half-fractional factorial design was 2^{n-1}. The design matrix, reported in Table 1, included 16 experiments, plus a centre point (exp. #17) replicated twice, allowing for the estimation of experimental error. The design space was constructed and analyzed using the Design-Expert® Software, Version 8.0.7.1 (Stat-Ease, Inc., USA).

2.3. Preparation of spray dried powders

2.5 g of amikacin sulphate raw material and the reported percentage of excipient (PEG-32 stearate) (Table 1) were dissolved in 60 mL of water at room temperature. Water and ethanol were added under stirring to obtain a final solution having the composition and concentration as reported in Table 1. The solutions obtained were spray dried using a Büchi Mini Spray Dryer B-290 (Büchi Labortechnik, Flawil, Switzerland) coupled to a B-296 dehumidifier, according to the process parameters reported in Table 1. The aspirator rate and atomizing air rate were kept constant at 90% of the total capacity and at 600 L/h, respectively. In addition, nozzle cleaning interval was adjusted at level 5 (one pressure blow every 7 s).

The spray dried powder was quantitatively recovered from the product collection vessel, weighed on an analytical balance (sensitivity 0.1 mg) (Mod. E50S, Gibertini, Italy) and expressed as percentage of the amount of solid dissolved in the sprayed solution. The dry product was stored at room temperature in a 25 mL cylindrical glass vial sealed with a rubber stopper and aluminium cap.

2.4. Agglomeration procedure

The spray dried powder was placed on the top of a stack of two sieves with nominal apertures of 600 μm and 106 μm, respectively (10 cm diameter sieves, Endecotts Ltd., London, UK); the final collector was added to the stack. The sieve stack was closed with the glass cover and vibrated for 5 min on a laboratory sieve shaker (amplitude 3; Analysette 3 Fritz model, Fritsch GMBH, Germany). Agglomerates retained between 600 μm and 106 μm were collected. The non-agglomerated powder in the collector was

Table 1
Matrix of half-fractional factorial design including the replicated centre point 17.

#	Factor 1 Drying temp. (°C)	Factor 2 Feed rate (ml/min)	Factor 3 Ethanol (% v/v)	Factor 4 Excipient (% w/w)	Factor 5 AMK conc. (% w/v)
1	130	2.0	20	0.0	3
2	160	2.0	20	0.0	1
3	130	5.0	20	0.0	1
4	160	5.0	20	0.0	3
5	130	2.0	10	0.0	1
6	160	2.0	10	0.0	3
7	130	5.0	10	0.0	3
8	160	5.0	10	0.0	1
9	130	2.0	20	1.0	1
10	160	2.0	20	1.0	3
11	130	5.0	20	1.0	3
12	160	5.0	20	1.0	1
13	130	2.0	10	1.0	3
14	160	2.0	10	1.0	1
15	130	5.0	10	1.0	1
16	160	5.0	10	1.0	3
17	145	3.5	15	0.5	2
17bis	145	3.5	15	0.5	2
17ter	145	3.5	15	0.5	2

Please cite this article in press as: Belotti, S., et al., Spray dried amikacin powder for inhalation in cystic fibrosis patients: A quality by design approach for product construction, Int J Pharmaceut (2014), http://dx.doi.org/10.1016/j.ijpharm.2014.05.055